NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 451

TOXICOLOGY AND CARCINOGENESIS STUDIES OF NICKEL OXIDE

(CAS NO. 1313-99-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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July 1996

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CONTENTS

ABSTRACT

NiO

NICKEL OXIDE

CAS No. 1313-99-1

Chemical Formula: NiO Molecular Weight: 74.71

Synonyms: Bunsenite; C.I. 77777; green nickel oxide; mononickel oxide; nickel monoxide; nickel oxide sinter 75; nickel protoxide; nickel (II) oxide; nickel (T+) oxide; nickelous oxide

Nickel oxide (NiO) "sinters" are used in stainless steel and alloy steel production. Nickel oxide was nominated by the National Cancer Institute to the NTP for testing because exposure to this form of nickel is prevalent in the nickel industry. Increased incidences of lung and nasal sinus cancers have occurred among workers in certain nickel refining facilities, and nickel oxide was studied as part of a class study of nickel compounds. Male and female F344/N rats and B6C3F$_1$ mice were exposed to nickel oxide (high temperature, green nickel oxide; mass median diameter 2.2 $\pm$ 2.6 μm; at least 99% pure) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in peripheral blood of B6C3F$_1$ mice exposed to nickel oxide for 13 weeks.

16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 1.2, 2.5, 5, 10, or 30 mg nickel oxide/m^3 (equivalent to 0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg nickel/m^3) by inhalation for 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of five male and five female rats were exposed to 0, 1.2, 5, or 10 mg/m^3 for tissue burden studies. All core study rats survived until the end of the study, final mean body weights of exposed male and female rats were similar to those of the controls, and there were no clinical findings related to nickel oxide exposure. Absolute and relative lung weights of male and female rats exposed to 10 or 30 mg/m^3 were significantly greater than those of the controls. Pigment particles in alveolar macrophages or within the alveolar spaces were observed in the lungs of exposed groups of males and females. Chronic-active inflammation and accumulation of macrophages in alveolar spaces of the lungs and hyperplasia in the respiratory tract lymph nodes were most severe in 10 and 30 mg/m^3 males and females. Hyperplasia of bronchial lymph nodes occurred in 30 mg/m^3 rats. Atrophy of the olfactory epithelium was observed in one male and one female exposed to 30 mg/m^3. The concentrations of nickel oxide in the lungs of exposed groups of rats were greater than those in the lungs of control groups (males, 42 to 267 μg nickel/g lung; females, 54 to 340 μg/g lung).

16-DAY STUDY IN MICE

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 1.2, 2.5, 5, 10, or 30 mg nickel oxide/m^3 by inhalation for 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of five male and

five female mice were exposed to 0, 1.2, 2.5, or 5 mg/m^3 for tissue burden studies. No exposure-related deaths occurred among core study mice, and final mean body weights of exposed male and female mice were similar to those of the controls. There were no chemical-related clinical findings. Pigment particles were present in the lungs of mice exposed to 2.5 mg/m^3 or greater. Accumulation of macrophages in alveolar spaces was observed in the lungs of 10 and 30 mg/m^3 males and females. The concentrations of nickel oxide in the lungs of exposed groups of mice were significantly greater than those in the lungs of control animals (males, 32 to 84 μg nickel/g lung; females, 31 to 71 μg/g lung).

13-Week Study in Rats

Groups of 10 male and 10 female F344/N rats were exposed to 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel oxide/m^3 (equivalent to 0, 0.4, 0.9, 2.0, 3.9, or 7.9 mg nickel/m^3) by inhalation for 6 hours per day, 5 days per week for 13 weeks. Additional groups of 18 male and 18 female rats were exposed to 0, 0.6, 2.5, or 10 mg/m^3 for tissue burden studies. No exposure-related deaths occurred among core study rats, final mean body weights of exposed male and female rats were similar to those of the controls, and no clinical findings in any group were related to nickel oxide exposure. Lymphocyte, neutrophil, monocyte, and erythrocyte counts; hematocrit values; and hemoglobin and mean cell hemoglobin concentrations in exposed rats were minimally to mildly greater than those of the controls; these differences were most pronounced in females. Mean cell volumes in exposed rats were generally less than those in the controls. Absolute and relative lung weights of exposed groups of males and females were generally significantly greater than those of controls.

Chemical-related nonneoplastic lesions were observed in the lungs of male and female rats exposed to concentrations of 2.5 mg/m^3 or higher, and the severity of these lesions generally increased with exposure concentration. Accumulation of alveolar macrophages, many of which contained black, granular pigment, was generally observed in all exposed groups of males and females, and increased incidences of inflammation occurred in males and females exposed to 2.5 mg/m^3 or higher. In addition, lymphoid hyperplasia and pigment occurred in the bronchial and mediastinal lymph nodes of 2.5, 5, and 10 mg/m^3 males and females.

The concentration of nickel oxide in the lungs of 0.6, 2.5, and 10 mg/m^3 males was greater than in the lungs of controls at 4, 9, and 13 weeks, and nickel continued to accumulate in the lung at the end of the 13-week exposures (4 weeks, 33 to 263 μg nickel/g lung; 9 weeks, 53 to 400 μg/g lung; 13 weeks, 80 to 524 μg/g lung).

13-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel oxide/m^3 by inhalation for 6 hours per day, 5 days per week for 13 weeks. Additional groups of six male and six female mice were exposed to 0, 0.6, 2.5, or 10 mg/m^3 for tissue burden studies. No exposure-related deaths occurred among core study animals, final mean body weights of exposed male and female mice were similar to those of the controls, and no clinical findings in any group were related to nickel oxide exposure. Hematocrit values and erythrocyte counts in 5 and 10 mg/m^3 females were minimally greater than those of the controls, as was the hemoglobin concentration in 5 mg/m^3 females. Absolute and relative lung weights of 10 mg/m^3 males and females were significantly greater than those of controls, and absolute and relative liver weights of 10 mg/m^3 males were significantly less than those of controls.

Accumulation of alveolar macrophages, many of which contained pigment particles, occurred in all groups of mice exposed to nickel oxide. Inflammation (chronic active perivascular infiltrates or granulomatous) occurred in 2.5, 5, and 10 mg/m^3 males and females. In addition, lymphoid hyperplasia and pigment occurred in the bronchial lymph nodes of males and females exposed to 2.5 mg/m^3 or higher.

The concentration of nickel in the lung was greater than that of controls in 0.6, 2.5, and 10 mg/m^3 males at 13 weeks (42 to 736 μg nickel/g lung).

2-YEAR STUDY IN RATS

Survival, Body Weights, Clinical Findings, and Hematology

Groups of 65 male and 65 female F344/N rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (equivalent to 0, 0.5, 1.0, or 2.0 mg nickel/m^3) by inhalation for 6 hours per day, 5 days per week for 104 weeks. Survival of exposed male and female rats was similar to that of the controls. Mean body weights of 1.25 mg/m^3 females and 2.5 mg/m^3 males and females were slightly lower than those of the controls during the second year of the study. No chemical-related clinical findings were observed in male or female rats during the 2-year study. No chemical-related differences in hematology parameters were observed in male or female rats at the 15-month interim evaluation.

Pathology Findings

Absolute and relative lung weights of 1.25 and 2.5 mg/m^3 males and females were significantly greater than those of the controls at 7 and 15 months. At 2 years, there were exposure-related increased incidences of alveolar/bronchiolar adenomas or alveolar/bronchiolar adenoma or carcinoma (combined) in males and females. Incidences of atypical alveolar epithelial hyperplasia in the lungs generally increased with increasing exposure concentration in male and female rats. Chronic inflammation of the lung was observed in most exposed rats at 7 and 15 months and at 2 years; the incidences in exposed males and females at 2 years were significantly greater than those in the controls, and the severity of the inflammation increased in exposed groups. The incidences of pigmentation in the alveolus of exposed groups of males and females were significantly greater than those of the controls at 7 and 15 months and at 2 years.

Pigmentation in the bronchial lymph nodes similar to that in the lungs was observed in all exposure groups with the exception of 0.62 mg/m^3 males and females at 7 months. Lymphoid hyperplasia was observed in the bronchial lymph nodes of 1.25 and 2.5 mg/m^3 males and females at 7 and 15 months, and the incidence at 2 years generally increased with exposure concentration.

At 2 years, there was an exposure-related increase in the incidence of benign pheochromocytoma in males and females. The incidences of benign pheochromocytoma and adrenal medulla hyperplasia in 2.5 mg/m^3 females and the incidence of benign or malignant pheochromocytoma (combined) in 2.5 mg/m^3 males were significantly greater than those in the controls.

Tissue Burden Analyses

Nickel concentrations in the lung of exposed rats were greater than those in the controls at 7 and 15 months (7 months, 173 to 713 μg nickel/g lung; 15 months, 262 to 1,116 μg/g lung), and nickel concentrations increased with increasing exposure concentration and with time.

2-YEAR STUDY IN MICE

Survival, Body Weights, Clinical Findings, and Hematology

Groups of 74 to 79 B6C3F_1 mice were exposed to 0, 1.25, 2.5, or 5 mg nickel oxide/m^3 by inhalation for 6 hours per day, 5 days per week for 104 weeks. Survival of exposed male and female mice was similar to that of the controls. Mean body weights of 5 mg/m^3 females were slightly lower than those of the controls during the second year of the study. No chemical-related clinical findings were observed in male or female mice during the 2-year study. No chemical-related differences in hematology parameters were observed in male or female mice at the 15-month interim evaluation.

Pathology Findings

At 2 years, the incidence of alveolar/bronchiolar adenoma in 2.5 mg/m^3 females was significantly greater than that of the controls, as was the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m^3 females. Generally, incidences of chronic inflammation increased with exposure concentration in males and females at 7 and 15 months. Bronchialization of minimal severity in exposed animals and proteinosis were first observed at 15 months. At 2 years, the incidences of chronic inflammation, alveolar epithelial hyperplasia, and proteinosis in exposed groups of males and females were significantly greater than those of the controls. The severity of chronic inflammation increased with exposure concentration in females, and proteinosis was most severe in 5 mg/m^3 males and females. Pigment occurred in the lungs of nearly all exposed

mice at 7 and 15 months and at 2 years, and the severity increased with exposure concentration.

Lymphoid hyperplasia occurred in two animals after 7 months; at 15 months, lymphoid hyperplasia occurred in males exposed to 2.5 and 5 mg/m^3 and in all exposed groups of females. At 2 years, lymphoid hyperplasia occurred in some control animals, but this lesion was still observed more often in exposed males and females and the incidence increased with exposure concentration. Pigmentation was observed in the bronchial lymph nodes of exposed males and females at 7 and 15 months and in nearly all exposed animals at 2 years.

Tissue Burden Analyses

Nickel concentrations in the lungs of exposed mice were greater than those in the controls at 7 and 15 months (7 months, 162 to 1,034 μg nickel/g lung; 15 months, 331 to 2,258 μg/g lung), and nickel concentrations increased with increasing exposure concentration and with time.

GENETIC TOXICOLOGY

No increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male or female mice exposed to nickel oxide.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity** of nickel oxide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign or malignant pheochromocytoma (combined) of the adrenal medulla. There was *some evidence of carcinogenic activity* of nickel oxide in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign pheochromocytoma of the adrenal medulla. There was *no evidence of carcinogenic activity* of nickel oxide in male B6C3F$_1$ mice exposed to 1.25, 2.5, or 5 mg/m^3. There was *equivocal evidence of carcinogenic activity* of nickel oxide in female B6C3F$_1$ mice based on marginally increased incidences of alveolar/bronchiolar adenoma in 2.5 mg/m^3 females and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m^3 females.

Exposure of rats to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung, lymphoid hyperplasia and pigmentation in the bronchial lymph nodes, and hyperplasia of the adrenal medulla (females). Exposure of mice to nickel oxide by inhalation for 2 years resulted in bronchialization, proteinosis, inflammation, and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Oxide

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Exposure concentrations	0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg nickel/m^3)	0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg nickel/m^3)	0, 1.25, 2.5, or 5 mg nickel oxide/m^3 (0, 1.0, 2.0, or 3.9 mg nickel/m^3)	0, 1.25, 2.5, or 5 mg nickel oxide/m^3 (0, 1.0, 2.0, or 3.9 mg nickel/m^3)
Body weights	2.5 mg/m^3 group slightly lower than controls	1.25 and 2.5 mg/m^3 groups slightly lower than controls	Exposed groups similar to controls	5 mg/m^3 group slightly lower than controls
2-Year survival rates	14/54, 15/53, 15/53, 12/52	21/53, 26/53, 20/53, 26/54	19/57, 23/67, 29/66, 23/69	41/64, 40/66, 42/63, 38/64
Nonneoplastic effects	Lung: chronic inflammation (28/54, 53/53, 53/53, 52/52); pigment (1/54, 53/53, 53/53, 52/52) Bronchial lymph node: lymphoid hyperplasia (0/52, 7/51, 10/53, 18/52); pigment (0/52, 45/51, 51/53, 51/52)	Lung: chronic inflammation (18/53, 52/53, 53/53, 54/54); pigment (0/53, 52/53, 53/53, 54/54) Bronchial lymph node: lymphoid hyperplasia (1/49, 5/50, 20/53, 13/52); pigment (0/49, 43/50, 52/53, 47/52) Adrenal medulla: hyperplasia (8/51, 12/52, 14/53, 22/53)	Lung: bronchialization (0/57, 24/67, 40/66, 40/69); proteinosis (0/57, 12/67, 22/66, 43/69); chronic inflammation (0/57, 21/67, 34/66, 55/69); pigment (0/57, 65/67, 66/66, 68/69) Bronchial lymph node: lymphoid hyperplasia (5/45, 18/56, 28/61, 33/62); pigment (0/45, 55/56, 61/61, 60/62)	Lung: bronchialization (0/64, 35/66, 39/63, 40/64); proteinosis (0/64, 8/66, 17/63, 29/64); chronic inflammation (7/64, 43/66, 53/63, 52/64); pigment (0/64, 64/66, 61/63, 64/64) Bronchial lymph node: lymphoid hyperplasia (14/54, 37/63, 40/59, 44/62); pigment (0/54, 58/63, 56/59, 60/62)
Neoplastic effects	Lung: alveolar/bronchiolar adenoma or carcinoma or squamous cell carcinoma (1/54, 1/53, 6/53, 4/52) Adrenal medulla: benign or malignant pheochromocytoma (27/54, 24/52, 27/53, 35/52)	Lung: alveolar/bronchiolar adenoma or carcinoma (1/53, 0/53, 6/53, 5/54) Adrenal medulla: benign pheochromocytoma (4/51, 7/52, 6/53, 18/53)	None	None
Uncertain findings	None	None	None	Lung: alveolar/bronchiolar adenoma (2/64, 4/66, 10/63, 3/64); alveolar/bronchiolar adenoma or carcinoma (6/64, 15/66, 12/63, 8/64)
Level of evidence of carcinogenic activity	Some evidence	Some evidence	No evidence	Equivocal evidence
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative in male and female mice		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

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TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on nickel oxide on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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Jerrold M. Ward, D.V.M., Ph.D., Principal Reviewer
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* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 29, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of nickel oxide received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, N.C.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of nickel oxide by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. The proposed conclusions were *some evidence of carcinogenic activity* in male and female F344/N rats, *no evidence of carcinogenic activity* in male B6C3F$_1$ mice, and *equivocal evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. Klaassen, a principal reviewer, agreed with the proposed conclusions. He asked why interim evaluations were conducted at 7 and 15 months. Dr. Dunnick replied that a large database exists for these time points, and they were chosen with the intent to examine the progression of lesions and lung nickel levels. Dr. Klaassen asked why Shirley's test was used for the statistical evaluation of nickel lung burden parameters. Dr. J.K. Haseman, NIEHS, explained that lung burden data are not normally distributed; therefore, a test using rank-based methods (Shirley's test) was considered to be most appropriate.

Dr. Ward, the second principal reviewer, agreed with the proposed conclusions for male and female rats and male mice. But, he suggested a conclusion of *no evidence of carcinogenic activity* for female mice based on the lack of dose response, the absence of any increase in neoplasm multiplicity, and the presence of statistical significance only for alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m^3 females. Dr. Ward also noted that although the increase in nickel lung burden measurements was dose related, neoplasm incidence was not. Dr. Haseman said that the lack of a dose response in 5 mg/m^3 females might have been due to significantly increased lung weights, adding that a significant correlation between increased lung weights and decreased lung neoplasm incidence was consistently observed in the three NTP nickel studies. Dr. Ward noted that in the present studies a high incidence of chronic inflammation of the lung was observed in control rats but not in control mice, and he asked if these spontaneous lesions were more specifically focal alveolar macrophages rather than chronic inflammatory lesions resulting from persistent toxins. Dr. M.R. Elwell, NIEHS, said the background inflammatory lesions in control animals were morphologically different from those in exposed animals and were primarily increases in the number of macrophages.

Because Dr. Reddy, the third principal reviewer, was unable to attend the meeting, Dr. L.G. Hart, NIEHS, read his review into the record. Dr. Reddy agreed with the proposed conclusions, although he believed the data for female mice supported a call of *some evidence of carcinogenic activity*. He expressed concern that the highest exposure level selected for rats (2.5 mg/m^3) was too low, and therefore the conclusion of *some evidence* was conservative.

Ms. D. Sivulka, executive director of the Nickel Producers Environmental Research Association, Inc. (NiPERA), commented on the discussion of evidence for nickel toxicity and carcinogenesis in humans and the presentation of the significance of findings relative to existing threshold limit values (TLVs). Ms. Sivulka said that because conclusions in the report were based on existing TLVs, an implication could be made that current regulations are not protective of workers exposed to nickel compounds. Ms. Sivulka discussed the cohorts of workers exposed to nickel compounds that have been examined, and she said that the information obtained from these examinations shows no evidence of nickel-related increases in the incidence of nonneoplastic lesions in workers exposed to low nickel levels.

Dr. Miller noted that TLVs are based to some degree on data obtained from animal studies. Dr. Russo asked if any of the human studies cited by NiPERA had corrected for confounding factors such as alcohol or tobacco exposure. Ms. Sivulka said they had not,

but added that the incidence of neoplasia in workers exposed to nickel compounds could not be attributed solely to factors such as cigarette smoking. Dr. Goldsworthy noted that nickel measurements were generally made without regard to form or species. Ms. Sivulka agreed, but added that NiPERA officials support exposure measurements and speciation analyses. Dr. Miller asked that information interpreting animal studies in terms of likely human exposure be added to the abstract of the Technical Report. Dr. R.A. Griesemer, NIEHS, said the report deals with a specific set of experiments and does not base conclusions on other animal or human studies. Dr. W.T. Allaben, NCTR, noted that the discussion section of the report is the appropriate area to speculate on such issues. He said it was the regulatory agencies' role to determine the significance of the nickel compound studies with regard to human risk.

Dr. Klaassen moved that the Technical Report on nickel oxide be accepted with the revisions discussed and with the conclusions as written for male and female rats, *some evidence of carcinogenic activity*; for male mice, *no evidence of carcinogenic activity*; and for female mice, *equivocal evidence of carcinogenic activity*. Dr. Goldsworthy seconded the motion, which was accepted unanimously with seven votes.